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Short Communication

Molecular and Serological Evaluation of *Neospora caninum* Infection in Dogs from a Rural Setting in Fars Province, Southern Iran

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Abstract

Background: Dogs, as the definitive host of *Neospora caninum*, are important in the epidemiology of this parasitic infection. We aimed to determine the prevalence of *N. caninum* infection in a dog population from a rural setting in Fars Province, Southern Iran, using a combination of molecular and serological techniques.

Methods: This cross-sectional study was carried out in Nov 2018 in three rural districts, Sar Mashhad, HosseinAbad, and Tolesaman located in Kazeroun Township in Fars province, southern Iran. Blood samples were taken from 60 stray and household dogs. Dogs' sera were tested for antibodies against *N. caninum*, using a *Neospora*-Modified Agglutination Test. Moreover, dogs' buffy coats were tested for *Neospora* DNA, using a molecular method.

Results: Anti-*Neospora* antibodies were detected in sera of 4 out of 60 dogs, corresponding to a seroprevalence rate of 6.7%. Out of 25 female dogs, 1 was seropositive and of 35 males, 3 were seropositive, yet the differences were not statistically significant. The infection was more prevalent in adult dogs (> 12 months), nevertheless, the differences between age and *Neospora* seropositivity was not statistically significant. *N. caninum* DNA was not detected in the buffy coat of any of the studied dogs.

Conclusion: Findings of the study indicate that *N. caninum* is a common infection in dogs in rural areas of Fars province in southern Iran. The infected dogs might be a potentially important source of *N. caninum* infection to livestock in the area.

Introduction

Neospora caninum is an important parasitic infection in dogs. The disease is clinically important in cattle, which may cause abortion in these animals (1, 2). As oocysts are rarely found in dogs feces, serological surveys remain the best approach to determine the prevalence of canine neosporosis (3).

Neospora is a common protozoan infection in dogs in different parts of the world, including Iran (3-6). The seroprevalence rate of *N. caninum* in dogs in Tehran, capital of Iran, was reported to be 33% in rural and urban dogs and 28% in household dogs (4, 5). A similar rate of infection (27%) has been reported for *Neospora* infection in dogs in Urmia, in West of Iran (7). Seroprevalence rate of *N. caninum* in dogs from Belgium was reported to be 11% and in dogs in a rural area in northeastern China was reported to be 20% (8, 9). The infected dogs might be a potentially important source of *N. caninum* infection to livestock (10).

Previous studies demonstrated an association between abortion in cattle and the presence of dogs in the environment (11, 12). In rural areas of Iran, domestic as well as stray dogs are in close contact with livestock, which increases the risk of transmission of *Neospora* to these animals. Serological and molecular studies of *Neospora* infection in dogs in Iran are limited.

We aimed to determine the extent of *N. caninum* infection in a dog population from a rural area in Fars Province, Southern Iran, using a combination of molecular and serological methods.

Materials and Methods

Study area and sampling

This cross-sectional study was carried out in Nov 2018 in three rural districts, Sar Mashhad, HosseinAbad, and Tolesaman located in Kazeroun Township in Fars province, southern Iran. Blood samples were collected from sixty stray and household dogs. Sera and buffy coat were isolated from the whole blood and stored at -20 ºC, until use.

Detection of anti-N. caninum antibodies

Sera samples were tested for *N. caninum* antibodies, using a *Neospora*-Modified Agglutination Test (MAT) ,which was previously validated for the detection of antibodies in cattle and dogs (13, 14). The Nc1 isolate of *N. caninum* was used in the MAT assay. Briefly, dogs' sera were added to the U bottoms 96 well microplate and diluted two-fold, starting from 1:5 up to 1:160 titer. Dog's sera with MAT titers of 1:5 or higher were intended positive for *N. caninum* antibodies. Negative and positive controls (from a dog, parasitologically confirmed for neosporosis) were included in each run of the experiment.

DNA extraction and Polymerase Chain Reaction (PCR)

The genomic DNA from all 60 dogs' buffy coat samples was extracted, using a blood Genomic DNA Extraction kit (Favorgene Biotech Corp, Taiwan) following the manufacturer's instructions. The extracted DNA was amplified by PCR, using the forward (NP6 5'-CAGTCAACCTACGTCTTCT-3') and reverse (NP21: 5'-GTGCGTCCAATCCTGTAAC-3') primers (15). The conventional PCR reactions were performed to amplify the Nc-5 gene of *N. caninum*. The PCR was performed in a 25-µL reaction volume contained 30 ng of template DNA, 12.5 µL of 2× Taq PCR mix (Amplicon, Odense, Denmark), 0.5 µL of each primer (10 pmol/ µL), and 11.5 µL of ddH_2O. The temperature profile of the PCR was one cycle of 95 °C for 5 min, followed by 30 cycles of 94 °C for 50 sec, 55 °C for 30 sec, and 72 °C for 50 sec, and one cycle of 72 °C for 4 min. *N. caninum* DNA as positive control and double-distilled water (DDW) instead of template

DNA as negative control were included in each run of the experiment. The PCR products were separated on 1.5% agarose gel and stained with GelRed nucleic acid gel stain (GelRed®, Biotium, CA, USA) for visualization under a BioDoc gel documentation System (UVP, Upland, CA, USA). The expected length of the amplified DNA fragment was about 328 bp.

Statistical analysis

SPSS (Ver. 18) was used for the statistical analysis. The association of various demographic characteristics and seropositivity to *Neospora* infection was verified, using the chi-square test.

Ethical approval

The study was approved by the Ethics Committee of Shiraz University of Medical Sciences (SUMS) and all the experiments were performed considering the Ethical Principles in Animal Research issued by SUMS.

Results

Anti-*Neospora* antibodies were detected in sera of 4 out of 60 dogs, corresponding to a seroprevalence rate of 6.7%. Out of 25 female dogs, one was seropositive and of 35 males, 3 were seropositive, yet the difference between gender and *Neospora* seropositivity was not statistically significant.

The dogs were 1 to 7 yr old and their mean age was 2.9 (±1.4) yr. Even though the infection was more prevalent in adult dogs (> 12 months), nevertheless the differences between age and *Neospora* seropositivity was not statistically significant. *N. caninum* DNA was not detected in the buffy coat of any of the studied dogs.

Discussion

Dogs are the definitive hosts of *N. caninum* and have an important role in the epidemiology of neosporosis (3). The seroprevalence of *N. caninum* in dogs varies in different areas of the world, depending on the study method, the number of studied animals, geographical location and population of stray or household dogs. Dogs fed with raw meat have a higher rate of *Neospora* infection than those that use ready-to-eat foods (16).

In the current study, a relatively low seroprevalence rate of *Neospora* infection was documented in dogs, from a rural area in southern Iran. The seroprevalence rate of *Neospora* infection reported here was lower than that of reported in other areas of Iran including Ahvaz in the southwest, Meshkin-Shahr in the Northwest, Urmia in the West, and Tehran, capital of Iran (4, 5, 7, 17). In most of these studies, immunofluorescence antibody assay (IFA) with a relatively low cut off is considered as seropositivity while in the current study a well defines specific modified agglutination test was utilized to assess the dogs' seropositivity to *Neospora*. This might have reduced the rate of seropositivity for *Neospora* in our study in comparison with the previous ones.

An increasing pattern of *Neospora* seropositivity with age has been reported in some of the epidemiological studies, being conducted in different areas of Iran as well as other areas of the world (4, 7, 17, 18). In the current study, a higher non-significant seroprevalence rate for *N. caninum* was observed in the older dogs.

The associations between sex and seropositivity to *N. caninum* infection has been shown in some studies, while in other studies such association has not been reported (4, 7, 9, 19). In the current study, no statistically significant difference was seen in the seroprevalence of *N. caninum* in female and male dogs.

It should be highlighted that a very low proportion of seropositive dogs are passing oocysts to the environment and the seropositivity is merely a reflection of exposure to *Neospora* (3). King et al., evaluated the presence of *N. caninum* oocyst in the stool of 132 dogs in Australia where oocyst was only detected in 1.5%

of the cases whereas antibodies to *N. caninum* were detected in 27% of the cases (19).

In the present study, none of the studied dogs was positive by the molecular method and no parasite DNA was detected in buffy coat of any of the dogs. This may indicate that none of the studied dogs had acute neosporosis. It may also show that the buffy coat might not be a suitable specimen for molecular detection of *Neospora* in dogs. The presence of *N. caninum* in different dog's tissues including the brain, skeletal muscle, liver, spleen and lymph nodes were evaluated in 42 stray dogs from Tehran, Iran where a prevalence of 35% was reported for *N. caninum* in the sampled dogs, using Nested-PCR (20). The highest rate of *N. caninum* infection has been found in skeletal muscle. This indicated that skeletal muscle is an appropriate sample for molecular assessment of *N. caninum* in dogs (20). Finally, the small number of the evaluated dogs is one of the shortcomings of this study.

Conclusion

Findings of the current study indicated that *N. caninum* is a common protozoan infection in dogs in the rural area of Fars province. Dogs infection warranty the transmission of *N. caninum* to cattle and subsequent abortion in these animals. A detailed study, focusing on *N. caninum* in cattle in the area is necessary to find effectively out the risk that infected dogs may present to livestock.

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Conflict of interest

The authors declare that there is no conflict of interest.

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